



Greater fatigability in knee-flexors vs knee-extensors after a standardized fatiguing protocol

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Abstract

The present study aimed to investigate the effects of a standardized fatiguing protocol on central and peripheral fatigue in knee-flexors and knee-extensors. Thirteen healthy men (age: 23 ± 3 years; height: 1.78 ± 0.09 m; body-mass: 73.6 ± 9.2 kg) volunteered for the present study. Maximal voluntary contraction (MVC), EMG activity, voluntary activation level (VAL) as an index of central fatigue and twitch potentiation as an index of peripheral fatigue were measured before and after the fatiguing protocol. The fatiguing protocol consisted of a 0.6 duty-cycle to exhaustion (6s isometric contraction, 4s recovery) at 70% MVC. After the fatiguing protocol, MVC decreased in both (ES=1.14) and knee-extensors (ES=1.14), and EMG activity increased in both knee-flexors (ES=2.33) and knee-extensors (ES=1.54). Decreases in VAL occurred in knee-flexors (ES=0.92) but not in knee-extensors (ES=0.04). Decreases in potentiation occurred in both knee-flexors (ES=0.84) and knee-extensors (ES=0.58). The greater central occurrence of fatigue in knee-flexors than in knee-extensors may depend on the different muscle morphology and coupled with a greater tolerance to fatigue in knee-extensors. The present data add further insight to the complicated knee-flexors-to-knee-extensors strength relationship and the mechanisms behind the different occurrence of fatigue.

Key-words: maximal voluntary contraction; voluntary activation level; potentiated twitch; EMG activity.

27 Introduction

28 Knee-flexors and knee-extensors are strictly and reciprocally connected since they act as antagonist
29 muscles for both knee and hip joints. Notwithstanding, because of the reported muscle strength
30 imbalance in favour of knee-extensors, possible repercussion on the knee-flexors injury risk exist
31 (Coratella, Bellin, Beato, & Schena, 2015; Coratella, Bellini, & Schena, 2016; Delextrat, Baker,
32 Cohen, & Clarke, 2013; Delextrat, Gregory, & Cohen, 2010; Rahnama, Reilly, Lees, & Graham-
33 Smith, 2003). Knee-flexors and knee-extensors strength was shown to be affected differently by
34 fatigue, since the maximal strength in knee-flexors **deteriorates** more compared to knee-extensors
35 (Coratella, Bellin, et al., 2015; Coratella et al., 2016; Delextrat et al., 2010; Rahnama et al., 2003).
36 This has practical relevance, given a greater incidence of knee-flexors injuries reported within the
37 last 15 minutes of a soccer match (Hawkins, Hulse, Wilkinson, Hodson, & Gibson, 2001). However,
38 how fatigue affects both knee-flexors and knee-extensors strength is still unclear and the
39 mechanisms behind the occurrence of fatigue need further and deeper investigation.

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41 Overall, fatigue is defined as a “progressive incapacity to maintain a given force continuously or
42 intermittently” (Enoka & Stuart, 1985) or “an exercise-induced reduction in maximal voluntary
43 muscle force” (Gandevia, 2001). The drop in maximal force depends on the neuromuscular capacity
44 to maximally recruit muscle activity, whose impairments **can derive from peripheral or central**
45 **mechanisms** (Gandevia, 2001). Such peripheral or central **occurrence** of fatigue can be investigated
46 using muscular or nervous electrical stimulations (Neyroud, Vallotton, Millet, Kayser, & Place,
47 2014; Rozand, Grosprêtre, Stapley, & Lepers, 2015). Among the possible markers, peripheral
48 fatigue is characterized by **a reduction** in resting-twitch elicited from electrical stimulations while
49 central fatigue can be assessed through the voluntary activation level (VAL) using the twitch
50 interpolation technique, which characterizes the ability to voluntarily activate most of the available
51 motor units (Gandevia, 2001; Rozand et al., 2015). Previous studies have investigated the
52 mechanisms behind fatigue in knee-extensors or knee-flexors using such a technique. In knee-

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3 53 extensors, inconsistent results have been found, since fatigue was mainly explained by central
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5 54 mechanisms after a rowing fatiguing task (Husmann et al., 2017), while no significant central
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7 55 fatigue was detected after an intermittent isometric exercise (Bachasson, Decorte, Wuyam, Millet,
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9 56 & Verges, 2016). On the contrary, the only study that investigated the knee-flexors occurrence of
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11 57 fatigue using the interpolated-twitch technique after a soccer-match simulation, showed that fatigue
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13 58 was mainly due to central factors (Marshall, Lovell, Jeppesen, Andersen, & Siegler, 2014).
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15 59 However, a direct comparison between knee-flexors and knee-extensors has not yet been performed.
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19
20 61 The studies that have previously investigated the effects of fatigue on knee-flexors and knee-
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22 62 extensors have shown some limitations. Consequently, these studies failed to provide an in-depth
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24 63 description of the mechanisms behind the occurrence of fatigue. Firstly, several studies used a
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26 64 soccer match simulation as a fatiguing protocol (Coratella, Bellin, et al., 2015; Delextrat et al.,
27
28 65 2010; Marshall et al., 2014; Rahnama et al., 2003). This includes several confounding factors, i.e.
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30 66 muscle activation, contraction modality and time under tension that may affect the occurrence of
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32 67 muscle fatigue, precluding a direct knee-flexors vs knee-extensors comparison. On the other hand, a
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34 68 study that used a comparable fatiguing task for both knee-flexors and knee-extensors did not
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36 69 investigate the occurrence of central or peripheral fatigue (Coratella et al., 2016). Consequently,
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38 70 although the afore-mentioned studies clearly reported that knee-flexors are more fatigable than
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40 71 knee-extensors, the causes of such a difference have not yet been elucidated. Therefore, the aim of
41
42 72 the current study was to investigate the central and peripheral contribution to both knee-flexors and
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44 73 knee-extensors fatigue after a standardized isometric fatiguing task. Since the higher prevalence of
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46 74 type-II fibres in knee-flexors compared to knee-extensors (Garrett, Califf, & Bassett, 1984), it was
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48 75 hypothesized that the knee-flexors would exhibit greater central and peripheral fatigue.
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54 77 **METHODS**
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79 **Participants**

80 Thirteen healthy men (age: 23(3) yrs; height: 1.78(0.09) m; weight: 73.6(9.2) kg) volunteered for
81 the present study. The participants were recreationally active in several sports but were not engaged
82 in any official competition or regular strenuous physical activity at that time. People with hip, knee
83 or ankle joint or muscular diseases were excluded from the present study. Similarly, the regular use
84 of drugs as well as the presence of any cardio-vascular and pulmonary diseases was considered as
85 exclusion criteria. The participants were instructed about any possible risks related to the present
86 procedures and they signed a written informed consent before their inclusion. The present study was
87 approved by the local Ethical Committee (CPP-Est-I-2016-A00511-50) and it was conducted
88 according to the recommendations of the Declaration of Helsinki (1975) for studies involving
89 human subjects.

90

91 **Experimental approach to the research question**

92 The current study was designed as a cross-sectional study. The participants were involved in two
93 separate sessions for knee-flexors or knee-extensors respectively. The two sessions were separated
94 by at least two days and **their order was randomized**. The participants avoided any form of
95 strenuous physical activity **at least** two days preceding the first session to the end of the study. The
96 fatiguing protocol consisted in a single-limb intermittent isometric exercise until exhaustion,
97 performed by the dominant limb (Figure 1A). The peripheral and central occurrence of fatigue was
98 investigated using electrical stimulations before and after the fatiguing task (Rozand et al., 2015),
99 **while the** mechanical and EMG signals were recorded throughout the fatiguing task. The study
100 design is depicted in Figure 1B.

101 **Please insert figure 1 here**

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103 **Strength measurement**

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3 104 The isometric maximal voluntary contraction (MVC) in both knee-flexors and knee-extensors was
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5 105 measured using a customized chair equipped with a strain gauge (Digital Transducer, MIE Medical
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7 106 Research, Leeds, United Kingdom) (Figure 1A). The participants sat on the chair at a hip angle of
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9 107 80° (0°=full extension) and were secured by two seatbelts. The belts secured the tested limb at 60°
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11 108 of knee flexion (0°=full extension) and the untested limb was immobilized by a fixed lever
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13 109 (Coratella, Milanese, & Schena, 2015). This angle was selected to avoid angles close to the full
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15 110 knee extension or flexion (*i.e.* knee-flexors or knee-extensors long muscle length, respectively),
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17 111 which would have affected the maximal voluntary activation (Doguet et al., 2017; Kluka et al.,
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19 112 2015). The upper-limbs were crossed against the chest. A strap was placed below the knee to avoid
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21 113 side-to-side movement and limit any force dispersion. Before the MVC assessment, the participants
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23 114 were familiarized with the procedures. Thereafter, they performed a warm-up protocol consisting of
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25 115 20 isometric contractions, separated by 10 s each, starting from a self-selected force and
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27 116 progressively increasing until the maximal volitional force was exerted. Then, two separate MVC
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29 117 trials were performed. If the difference between the two trials exceeded 5%, further trials were
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31 118 performed. Each trial lasted 4 s and was separated by 3 min of passive recovery. Immediately after
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33 119 the fatiguing protocol, one MVC was performed. Lastly, the knee-flexors-to-knee-extensors MVC
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35 120 ratio was calculated and inserted in the data analysis. The operators strongly encouraged the
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37 121 participants to “push” or “pull” as hard as they could to reach their maximal force on each trial. All
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39 122 force signals were recorded at 2 kHz using an AD conversion system (LabChart 8, ADInstruments,
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41 123 Sydney, Australia).

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48 125 **Experimental fatigue protocol**

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50 126 The participants sat on the customized chair used for testing MVC, with the upper-limbs crossed
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52 127 against their chest and the strap placed below the knee. The knee-extensors and knee-flexors MVC
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54 128 was used to individually tailor the fatiguing protocol. This consisted of a duty-cycle of 6 s isometric
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56 129 knee extension or flexion and 4 s of recovery until exhaustion (Conchola, Thiele, Palmer, Smith, &
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Thompson, 2015). During the isometric exercise, the participants were required to hold a horizontal line corresponding to 70% of the knee-extensors or knee-flexors MVC on a monitor displaying the force exerted in real time, as previously used (Boccia, Coratella, et al., 2016; Boccia, Dardanella, Coratella, et al., 2015; Boccia, Dardanella, et al., 2016; Boccia, Dardanella, Rinaldo, et al., 2015). The fatiguing protocol ended when the participants were not able to hold the line for two consecutive six-second contractions, despite their maximal efforts. The operators strongly encouraged the participants to resist as long as possible. Rating of perceived exertion was then recorded using a 0-10 Borg scale (Borg & Kaijser, 2006). Similarly, the number of repetitions to exhaustion performed during the fatiguing protocol for knee-flexors or knee-extensors was recorded.

EMG measurements

The activation of knee-flexors and knee-extensors were assessed using surface EMG signals recorded from *vastus lateralis* and the long head of *biceps femoris*, respectively (Conchola et al., 2015). The skin was shaved, abraded and cleaned with isopropyl alcohol to ensure low impedance ($< 5 \text{ k}\Omega$). The electrodes were placed over each muscle belly following the SENIAM recommendations (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). The EMG signals were acquired using a wireless system (Trigno, Delsys Inc., USA). Before placing the sensors, an EMG signal-inspection on both knee-extensors and knee-flexors was performed to identify their best position (Beretta Piccoli et al., 2014). The EMG signals were amplified with a bandwidth frequency ranging from 0.3 Hz to 1 kHz at a sampling frequency of 2 kHz using the Powerlab data acquisition system (LabChart 8, ADInstruments, Sydney, Australia). To avoid any possible undesired movement or friction of the electrodes placed on knee-flexors, the participants sat on a tunnel-shaped foam board located under the thigh.

Muscle stimulation

The electrical stimulations were evoked on *biceps femoris* or *vastus lateralis* at baseline, during and 3 s after each MVC. Such a procedure was repeated before and after the fatiguing task. Direct muscle stimulation was applied to *biceps femoris* or *vastus lateralis* using pre-gelled surface electrodes (8 x 6 cm, Medicompex SA, Ecublens, Switzerland). To stimulate *biceps femoris*, the anode was placed above the popliteal fossa and the cathode below the gluteal fold. To stimulate *vastus lateralis*, the cathode was placed 2-to-3 cm above the superior aspect of the patella and the anode 4-to-5 cm below the inguinal fold. Doublet electrical stimulations were delivered by a high-voltage stimulator (Digitimer DS7AH, Welwyn Garden City, UK), which consisted of rectangular pulses (pulse duration: 1 ms) with an inter-pulse duration of 10 ms (100 Hz). Before each MVC, the participants were familiarized with the stimulations. The doublet stimulations were used both at rest and superimposed during each MVC. The stimulation intensity was gradually increased by 10 mA increments until a plateau in single-twitch force was reached. Then, 130% of such a voltage-intensity was used to achieve the supra-maximal intensity during the experimental session. For each MVC, three doublet stimulations at the supra-maximal intensity with an inter-pulse duration of 10 ms (100 Hz) were evoked: at rest before, during (superimposed) and after (potentiated doublet) the MVC. The superimposed doublet was manually triggered once the plateau of the MVC was reached. The potentiated doublet was evoked 3 s after relaxation.

Data analysis

During the MVCs, EMG signal was quantified as root mean square (RMS) amplitude for the 500 ms preceding the doublet stimulation. During the fatiguing protocol, RMS was calculated at 3 s for each isometric contraction during a 500 ms period. The time course of EMG signals during the fatiguing protocol was grouped as 25th, 50th, 75th and 100th percentile of the number of repetitions. The peak force of each mechanical response to doublet stimulations evoked at rest, during (superimposed twitches) and after MVC (potentiated twitches) was measured and analysed. The superimposed doublet was measured as the peak-to-peak mechanical twitch over the force-plateau

developed before the stimulation. Each twitch was measured manually after a careful selection and inspection of the time periods before and after the stimulations. Muscle potentiation was estimated through the ratio of the potentiated twitch divided by the resting twitch. The interpolated twitch technique was used to quantify maximal voluntary activation level (VAL) during the MVCs (Allen et al. 1995). The amplitude of each superimposed and potentiated doublet twitch was then used in the following formula: $VAL = (1 - (\text{superimposed doublet force} / \text{potentiated doublet force})) \times 100$.

Statistical analysis

The statistical analysis was performed using a statistical software (SPSS 20, IBM, USA). The normality of data was checked using the Kolmogorov-Smirnov test and all distributions were normal. The difference in the number of repetitions and rating of perceived exertion was analysed using a pairwise, two-tailed T-test. A two-way repeated-measures ANOVA was used to calculate differences in MVC, VAL, potentiation and EMG over time (two levels: pre and post) and muscle (two levels: knee-flexors and knee-extensors). Post-hoc analysis was then performed using Bonferroni's correction. A two-way repeated-measures ANOVA was performed to calculate differences in EMG activity during the fatiguing protocol over time (four levels: 25th, 50th, 75th and 100th percentiles) between knee-flexors and knee-extensors. Significance was set at $p < 0.05$. Data are shown as mean(SD). Changes are shown as mean change, with lower and upper bounds of confidence interval (CI95%). Effect-size (ES) was calculated and interpreted according to Cohen's recommendations: $< 0.20 = \text{trivial}$; $0.21-0.49 = \text{small}$; $0.50-0.79 = \text{moderate}$; $0.80-1.19 = \text{large}$; $\geq 1.20 = \text{very large}$.

Results

The number of repetitions to exhaustion was higher in knee-extensors than in knee-flexors [49(9) and 22(6) respectively, $p = 0.028$]. No difference was observed in the rating of perceived exertion at the end of both fatiguing protocols [knee-flexors 9.0(1.0), knee-extensors: 9.0(0.6), $p = 1.000$].

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5 208 Greater MVC was observed in knee-extensors than in knee-flexors, both before [181(41) and
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7 209 129(28.6) Nm, $p < 0.001$] and after the fatiguing protocol [132(36) and 94(27) Nm, $p = 0.002$].
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9 210 Similarly, no difference in knee-flexors-to-knee-extensors MVC ratio was found before and after
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11 211 the fatiguing protocol [0.72(0.12) and 0.73(0.21) A.U., $p = 0.735$]. At baseline, greater VAL was
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13 212 found in knee-flexors than in knee-extensors [83(12) and 76(12)%, $p = 0.041$].
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15 213 No interaction was found for MVC ($p = 0.791$). After the fatiguing protocol, both knee-flexors [-
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17 214 35(20) Nm, CI95% -51. to -17, ES = 1.14] and knee-extensors [-49(25) Nm, CI95% -75 to -23, ES
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19 215 = 1.14] showed decreases in MVC (Figure 2A). No interaction was found for EMG RMS ($p =$
20
21 216 0.654). Increases in EMG RMS were found in both *biceps femoris* [37(12)%, CI95% 22 to 49, ES =
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23 217 2.33] and *vastus lateralis* [51(33)%, CI95% 38 to 64, ES = 1.54] (Figure 2B). On the contrary, an
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25 218 interaction was found for VAL ($p = 0.026$). After the fatiguing protocol, a decrease in VAL was
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27 219 observed in knee-flexors [-12.8(4.2)%, CI95% -22.1 to -3.8, ES = 0.92] but not in knee-extensors [-
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29 220 3.6(4.4)%, CI95% -13.2 to 6.0, ES = 0.04] (Figure 2C).
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32 221 A significant decrease in potentiated doublet twitch amplitude was found, with no interaction
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34 222 between each muscle group ($p = 0.792$). The potentiated twitch in knee-extensors was 83(20) Nm
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36 223 and 60(7) Nm before and after the fatiguing protocol ($p = 0.001$). The potentiated twitch in knee
37
38 224 flexors was 74(12) Nm and 34(8) Nm, before and after the fatiguing protocol ($p < 0.001$).
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40 225 Regarding potentiation, no interaction was found ($p = 0.138$). Decreases in potentiation were
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42 226 observed both in knee-flexors [-46.9(17.1)%, CI95% -84.2 to -9.6, ES = 0.84] and in knee-
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44 227 extensors [-15.2(6.2)%, CI95% -28.4 to -1.9, ES = 0.59] (Figure 2D).
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52 230 No interaction was found for EMG RMS during the fatiguing protocol ($p = 0.155$). Compared to
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54 231 baseline, increases in EMG RMS were observed in *biceps femoris* after the 25th [34.3(6.5)%, CI95%
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56 232 11.7 to 56.9, ES = 2.23], 50th [56.6(13.4)%, CI95% 10.7 to 102.6, ES = 2.13], 75th [80.5(15.6)%,
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CI95% 26.8 to 134.1, ES = 2.60] and 100th [90.5(23.9)%, 8.6 to 172.5, ES = 1.96] percentile of the number of repetitions. Compared to baseline, increases in EMG RMS were observed in *vastus lateralis* after the 50th [33.4(7.2)%, CI95% 8.7 to 58.1, ES = 1.43], 75th [53.0(9.7)%, CI95% 19.7 to 86.3, ES = 1.57] and 100th [71.4(14.5)%, CI95% 21.6 to 121.2, ES = 1.86] but not after the 25th [9.1(4.3)%, CI95% -5.6 to 23.9, ES = 0.27] percentile. Compared to *vastus lateralis*, higher EMG RMS resulted in *biceps femoris* after the 25th [25.1(8.2)%, CI95% 7.2 to 43.1, ES = 1.31] and a strong trend resulted after the 75th percentile [27.4(12.8)%, CI95% -0.4 to 55.4, ES = 0.56]. No difference resulted after the 50th [23.2(12.2)%, CI95% -3.4 to 49.9, ES = 0.63] and the 100th [19.1(24.3)%, -33.8 to 72.0, ES = 0.27] percentile. (Figure 3)

Please insert figure 3 here

Discussion

To the best of the authors' knowledge, this is the first study that has investigated the central and peripheral occurrence of fatigue in knee-flexors and knee-extensors after a standardized fatiguing protocol. The current results showed that the reductions in isometric MVC, as well as the changes in EMG RMS and peripheral fatigue, were similar in both knee-flexors and knee-extensors after the fatiguing exercise. However, knee-extensors performed more repetitions before exhaustion than knee-flexors. This difference was accompanied by an evidence of central fatigue observed in knee-flexors only, as highlighted by the decrease in VAL. In addition, EMG activity during the fatiguing protocol increased over time both in *biceps femoris* and in *vastus lateralis*, but the increment occurred earlier in *biceps femoris*.

Very large decreases in isometric MVC were observed after the fatiguing protocol both in knee-flexors and in knee-extensors. While it is well known that fatigue affects the maximum muscle force production (Gandevia, 2001), the testing modality seems to influence the extent of strength loss. Indeed, after different fatiguing protocols, similar strength decrements were observed in knee-flexors and knee-extensors when measured in concentric (Coratella, Bellin, et al., 2015; Delestrat et

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3 259 al., 2010) or isometric (Coratella et al., 2016) modality. Consistently, the present results showed no
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5 260 difference in the isometric knee-flexors-to-knee-extensors MVC ratio, confirming the similar
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7 261 decreases in MVC. In contrast, when force was measured in eccentric modality, greater decrements
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9 262 were reported in knee-flexors compared to knee-extensors (Cohen, Zhao, Okwera, Matthews, &
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11 263 Delextrat, 2014; Coratella, Bellin, et al., 2015; Delextrat et al., 2013, 2010). The unique neural
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13 264 control of the eccentric contraction may account for such a difference. Indeed, earlier type-II fibre
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15 265 recruitment was reported in eccentric compared to concentric contraction (Duchateau & Enoka,
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17 266 2008; Roger M Enoka, 1996). Therefore, due to the different fibre type distribution in knee-flexors
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19 267 and knee-extensors, it can be argued that the higher type-II fibre prevalence in knee-flexors may
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21 268 result in a greater fatigability when tested in eccentric modality (Coratella, Bellin, et al., 2015).
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23 269 Thus, the similar decreases in MVC **shown here** can be consistently explained by the current
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25 270 isometric testing modality, which does not account for the muscle fibre type prevalence.
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31 272 Importantly, the previous studies that investigated the fatigue responses in knee-flexors or knee-
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33 273 extensors used running- or sprinting- or change of direction-based fatiguing protocols (Coratella,
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35 274 Bellin, et al., 2015; Delextrat et al., 2010; Rahnema et al., 2003). Such protocols were not matched
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37 275 for the amount of work **or** the intensity of effort. Consequently, the knee-flexors or knee-extensors
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39 276 muscle activity and amount of work performed could have been different, possibly leading to
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41 277 greater strength loss in knee-flexors. In the present study, the standardized fatiguing protocol
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43 278 resulted in *very large* decreases in isometric MVC in both knee-flexors and knee-extensors. Lastly,
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45 279 it must be acknowledged that due to the longer duration of the knee-extensors fatiguing task, the
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47 280 participants may have lost motivation and sub-maximally performed the MVCs (Place, Maffiuletti,
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49 281 Ballay, & Lepers, 2005). However, the similar RPE **score recorded at the end of the** fatiguing
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51 282 protocol in both knee-flexors and knee-extensors might lead to exclude such a confounding factor.
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284 Concurrently with the decreases in MVC, *large* and *very large* increments in EMG RMS were
285 observed in *biceps femoris* and *vastus lateralis* respectively after the fatiguing protocol. The
286 increments in EMG activity reflect the enhancements in the central drive to compensate for the loss
287 of motor unit activation induced by peripheral and spinal mechanisms (Fuglevand, Winter, & Patla,
288 1993; Garland, Enoka, Serrano, & Robinson, 1994). The current results are in agreement with a
289 previous study that reported increases in EMG activity during an intermittent isometric exercise
290 (Hermans & Spaepen, 1997). However, even if both *biceps femoris* and *vastus lateralis* showed
291 similar increases in EMG activity, an earlier fatigability was observed in *biceps femoris*.
292 Accordingly, during a standardized concentric-only fatiguing exercise, knee-flexors resulted in
293 earlier strength decrements compared to knee-extensors (Coratella et al., 2016). The higher number
294 of repetitions to exhaustion in knee-extensors observed here is also in line with the above-
295 mentioned study. However, since the EMG activity was not measured, a direct comparison cannot
296 be made. Both the earlier increment in EMG activity during the fatiguing protocol and the lower
297 number of repetitions to exhaustion suggest that knee-flexors could be more prone to fatigue
298 compared to knee-extensors. Importantly, knee-extensors were presently tested and fatigued at a
299 shorter muscle length compared to knee-flexors, due to the fixed seated position. In a previous
300 direct comparison, muscles at shorter vs longer length were shown to be more fatigue-resistant
301 (Place et al., 2005). Consequently, the longer knee-flexors muscle length may have contributed to
302 decrease their endurance capacity.

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304 Although both knee-flexors and knee-extensors showed decrements in MVC and increments in
305 EMG RMS, different mechanisms seem to be involved. Indeed, *large* decrements in VAL occurred
306 in knee-flexors (Marshall et al., 2014) but not in knee-extensors. The previous studies that evaluated
307 the occurrence of central fatigue in knee-extensors reported inconsistent outcomes. While
308 decrements in central drive were previously shown after repeated all-out sprints (Hureau, Ducrocq,
309 & Blain, 2016) or constant-load cycling (Thomas, Elmeua, Howatson, & Goodall, 2016; Weavil,

310 Sidhu, Mangum, Richardson, & Amann, 2016), other studies did not report any change after
311 intermittent (Bachasson et al., 2016) or continuous isometric knee-extensors fatiguing task
312 (Marshall, Finn, & Siegler, 2015). However, these two studies recruited endurance- (Bachasson et
313 al., 2016) or strength-trained (Marshall et al., 2015) participants. Since trained people could be
314 more fatigue-resistant, a possible greater central tolerance to fatigue may have been developed
315 (Marshall et al., 2015). Similarly, it may be argued that the baseline characteristics (fatigue-resistant
316 muscle fibre phenotype) in knee-extensors and their primary role in endurance daily activities (e.g.,
317 standing or walking) compared to knee-flexors may have increased their tolerance to central fatigue
318 (Bachasson et al., 2016). Accordingly, after the same standardized fatiguing task, knee-extensors
319 may have been less affected than knee-flexors. Importantly, the greater VAL shown in knee-flexors
320 suggests that long muscle length may have increased it (Doguet et al., 2017; Kluka et al., 2015).
321 However, this is in contrast with another study that found similar VAL at short vs long knee-
322 extensors muscle length (Place et al., 2005). Consequently, it may be speculated that the different
323 morphology in knee-flexors vs knee-extensors may have played a major role in muscle fatigue
324 compared to the different muscle length. However, the use of additional measurements (e.g.
325 transcranial stimulations) could have added further insight to the occurrence of central fatigue.

327 In the present study, the decreases in doublet twitch amplitude in both knee- flexors and
328 knee-extensors accounted for a similar global peripheral fatigue (Rozand et al., 2015). A closer look
329 at the mechanisms, such as the change in potentiation, showed that the global fatigue can originate
330 from the impairment in Ca^{2+} releasing process during a MVC (Place et al., 2005). In addition, such
331 a peripheral fatigue can also derive from impairments in the excitation-contraction coupling,
332 changes in cross-bridge properties or metabolic alterations (Allen, Lamb, & Westerblad, 2008). The
333 current study showed that knee-flexors (*very largely*) and knee-extensors (*moderately*) were both
334 affected by peripheral fatigue, contrarily to what was hypothesized previously. Changes in
335 peripheral fatigue were consistently reported in knee-extensors after isometric exercise (Bachasson

et al., 2016; Marshall et al., 2015), as well as after repeated all-out sprints (Hureau et al., 2016) or constant load to exhaustion in cycling protocols (Thomas et al., 2016). On the contrary, the only study that measured peripheral fatigue in knee-flexors detected no significant change (Marshall et al., 2014). However, in the latter study, the authors used a fatiguing protocol that simulated a soccer match, which included sprinting and change of direction. During such activities, knee-extensors have a greater role as primary movers compared to knee-flexors and this may result in a preservation of knee-flexors from further peripheral fatigue (Marshall et al., 2014). In contrast, the present procedures used a standardized protocol for both knee-flexors and knee-extensors. Therefore, the similar occurrence of peripheral fatigue (although with a different extent) may have derived from the comparable fatiguing protocol used here.

The present study comes with some acknowledged limitations. Firstly, only two muscles (*biceps femoris* and *vastus lateralis*) were selected to represent the knee-flexors and knee-extensors EMG activity respectively (Conchola et al., 2015). Since the complex morphology in knee-flexors and knee-extensors, further investigations on additional muscles are recommended. Secondly, the spinal contribution was not investigated. However, such a procedure is difficult to assess in both knee-flexors and knee-extensors. Therefore, to be consistent with previous procedures, the spinal contribution was not included here (Bachasson et al., 2016; Marshall et al., 2014). Furthermore, although the same standardized fatiguing protocol and strength measurement were used to avoid any possible confounding factor, different protocols and measurements might have resulted in different outcomes. Additionally, the present fatiguing protocol tested knee-flexors and knee-extensors at different muscle lengths. However, although the greater central activation reported at long vs short muscle length (Doguet et al., 2017), the rate of muscle fatigue was not affected (Guex, Degache, Gremion, & Millet, 2013).

In conclusion, a standardized intermittent sub-maximal isometric fatiguing protocol induced similar decreases in isometric MVC in both knee-flexors and knee-extensors. In addition, despite the earlier increase in EMG RMS in *biceps femoris*, both *biceps femoris* and *vastus lateralis* showed large increments in EMG RMS after the fatiguing protocol. However, notwithstanding the similar peripheral fatigue, only knee-flexors showed a large reduction in central motor commands.

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3 491 **Figure Captions**

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5 492 **Figure 1.** The experimental set-up for the knee flexion and the knee extension fatiguing protocols is
6
7 493 shown. **A.** Schema of the experimental setup with the participants positioning. **B.** Schematic
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9 494 description of the experimental design used for both knee-flexors and extensors. The vertical arrows
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11 495 depict stimulations used to evoke the twitches.

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16 497 **Figure 2.** Pre-post changes in MVC (A), EMG (B), VAL (C) and potentiation (D) in knee-
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18 498 extensors and knee-flexors.

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20 499 * = $p < 0.05$ in pre-post comparisons.

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22 500 **Figure 3.** Time course of the EMG activity in *vastus lateralis* and *biceps femoris* during their
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24 501 fatiguing protocols.

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26 502 * = $p < 0.05$ compared to baseline EMG activity

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28 503 # = $p < 0.05$ compared to *vastus lateralis* EMG activity.

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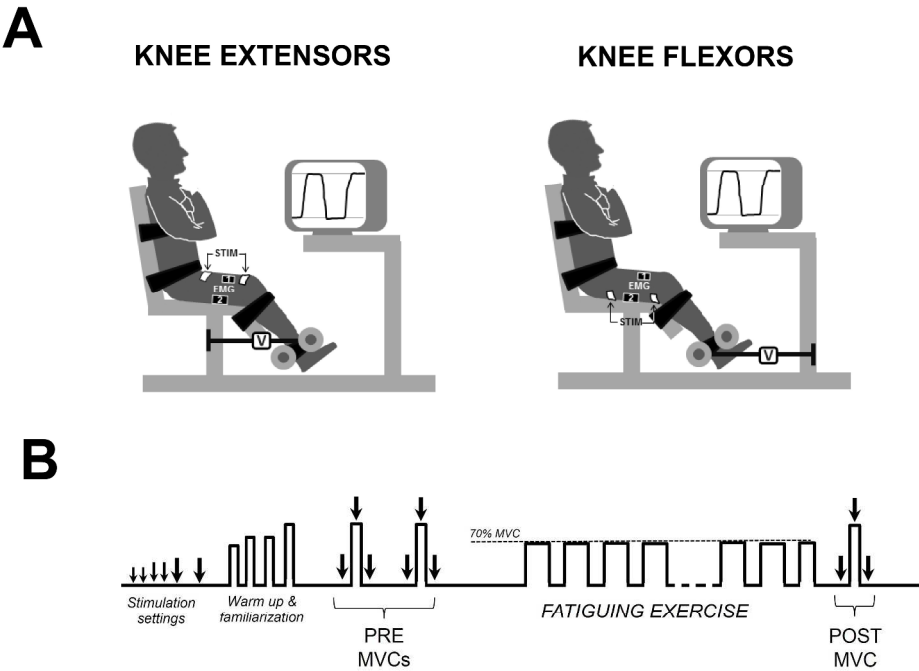


Figure 1. The experimental set-up for the knee flexion and the knee extension fatiguing protocols is shown. A. Schema of the experimental setup with the participants positioning. B. Schematic description of the experimental design used for both knee-flexors and extensors. The vertical arrows depict stimulations used to evoke the twitches.

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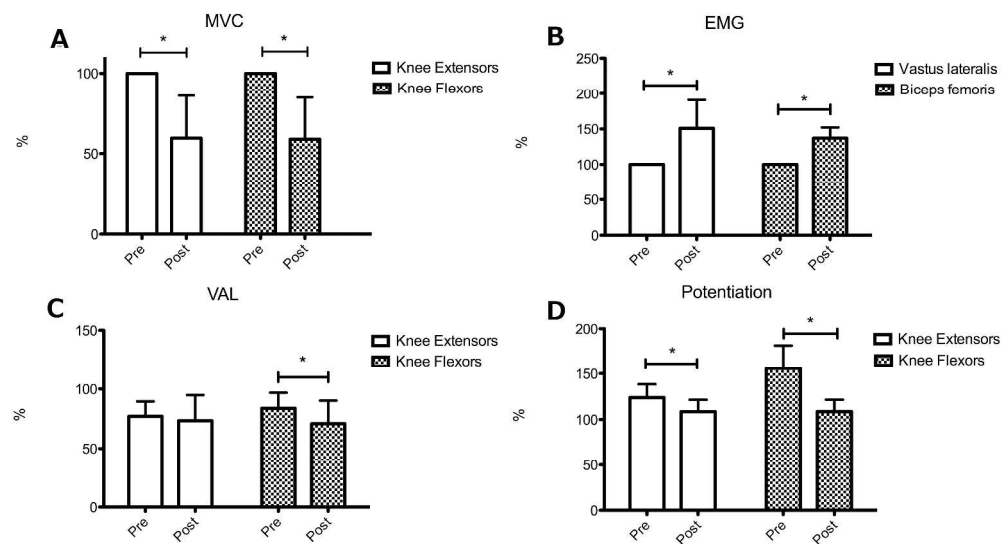


Figure 2. Pre-post changes in MVC (A), EMG (B), VAL (C) and potentiation (D) in knee-extensors and knee-flexors.

* = $p < 0.05$ in pre-post comparisons.

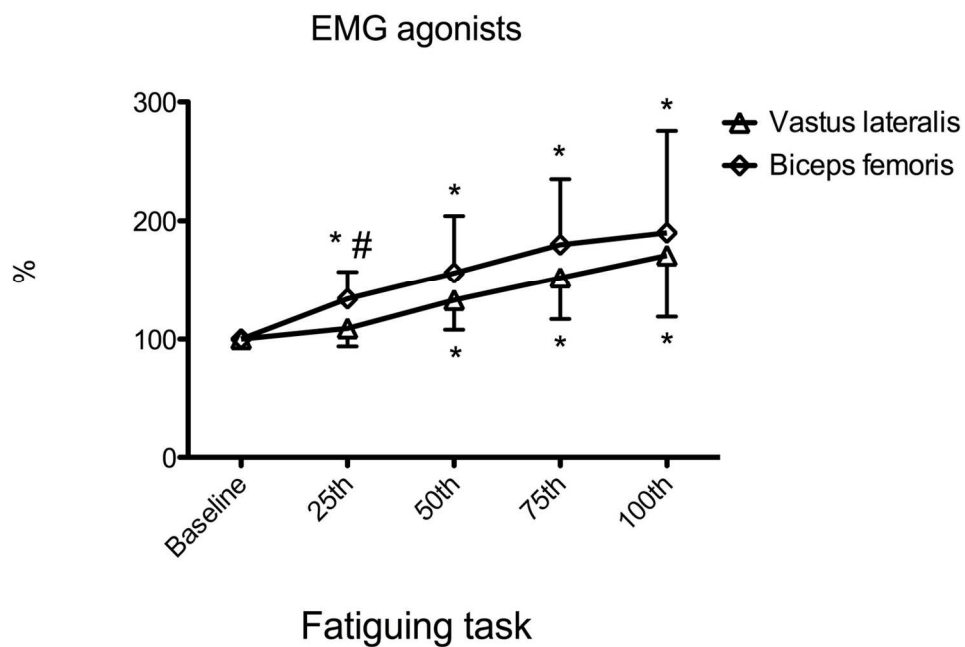


Figure 3. Time course of the EMG activity in vastus lateralis and biceps femoris during their fatiguing protocols.

* = $p < 0.05$ compared to baseline EMG activity
= $p < 0.05$ compared to vastus lateralis EMG activity.

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